STAIN TECHNOLOGY

VOLUME 38 MAY 1963 NUMBER 3

FUCHSIN STAINING WITH NaOH CLEARING FOR LIGNIFIED ELEMENTS OF WHOLE PLANTS OR PLANTS ORGANS

CH. FUCHS, *Laboratoire de Biologie Végétale Appliquée, Muséum National d'Histoire Naturelle, 61 rue Buffon, Paris 5e, France*

ABSTRACT. **Three methods that are adapted to the various consistencies of plants are as follows: 1. Samples are placed for 10–14 hr at 60° C in a 1% aqueous solution of basic fuchsin, to which 10 gm of solid NaOH per 100 ml are added. 2. Samples when taken out of 95% alcohol are placed in a 1% solution of basic fuchsin in 95% alcohol for 24 hr; after washing in water, they are placed in a 15% solution of NaOH at 60° C until cleared. 3. Samples are placed in a 15% aqueous solution of NaOH at 60° C until cleared, then for 24 hr at 60° C in 15% NaOH containing basic fuchsin. After being stained and cleared by one of these three methods, the samples are rinsed in water, dehydrated and then passed into a mixture of absolute alcohol and concentrated HCl (3:1) for 1–15 min, rinsed in absolute alcohol, cleared in xylene and mounted in Canada balsam. The lignified tissues appear red; the others, transparent.**

The study of the vascular system and the young organs of plants has been carried out by using not only anatomical and cytological methods, but also by means of clearing techniques (Brunotte 1900, Gabriel and Pelissier 1936, Debenham 1939, Pelissier 1939, Foster 1949, Wilson 1950, Kasapligil 1951, Leben 1952, Pray 1954, de Sloover 1958). During the course of an experimental study on the tracheogenesis of the leaf of *Lupinus albus* L., we attempted to use these methods, but all of them proved to have the disadvantage of slightly staining the hairs and the parenchymal tissue of this species, thus considerably impeding observation to any appreciable depths in the young plantlet. Hence, we developed a clearing and staining method which stains only the lignified or suberous elements, with the others remaining perfectly transparent.

The principle of this method is to stain the lignin by means of basic fuchsin and to carry out the clearing process with NaOH after the staining; but it is also possible to treat specimens with NaOH and fuchsin in the same solution. Using these possibilities as a basis, the practical applications can be varied; therefore, we shall describe three different methods.

PROCEDURES

Method No. 1. This sequence is suitable for plants of a soft consistency.

1. Place the samples in 80% alcohol for several days.
2. Rehydrate slowly.
3. Place for 10–14 hr at 60° C in a solution preparated in the following manner: Dissolve 1 gm of basic[1] fuchsin in 100 ml of boiling water. Do not filter, but after the solution is allowed to cool, add 10 gm of solid NaOH.

[1] We used "bacteriological basic fuchsin" R.A.L.—Kuhlmann for our tests, but basic fuchsins from other sources gave the same results.

Biotech Histochem Downloaded from informahealthcare.com by Karolinska Institutet University Library on 06/08/14
For personal use only.

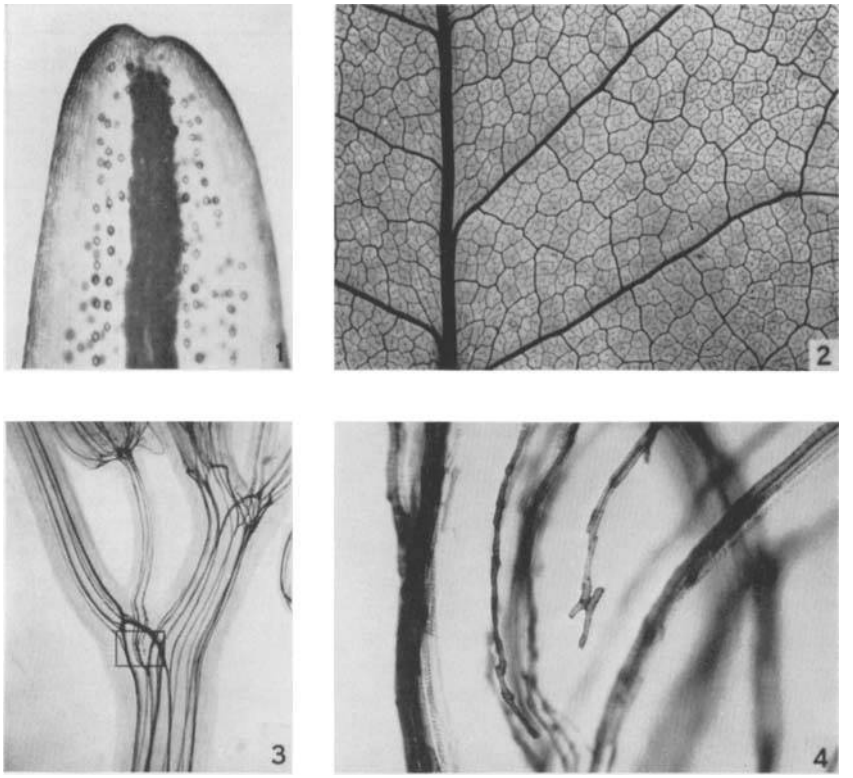

FIG. 1. *Abies lowiana* Murr., leaf; method No. 3 with counterstaining by fast green FCF. × 38

FIG. 2. *Rhododendron catawbiense* Michx., leaf; method No. 2 and fast green FCF. × 4.5

FIG. 3. *Tropaeolum majus* L. stem, petioles, pedicel and part of flower; method No. 1 and fast green FCF. × 4.8

FIG. 4. Enlarged view of part of Fig. 3. × 48

4. Wash 12 hr in water that is changed frequently. The sample should finally be a greyish-yellow color; the lignified elements, red.
5. Dehydrate by passing through graded alcohols (50, 70, 95%) and by replacing the alcohol by one of a higher degree only when it no longer becomes colored. This operation should last at least 12 hr since alcoholic dehydration plays an important part in the clearing process.
6. Pass into absolute alcohol 1–2 hr, depending on the size of the sample.
7. Place in a mixture of absolute alcohol and concentrated HCl (3:1). The lignified tissues that were red now become dark green and the sample should remain in this solution for sufficient time to allow the other tissues to become bleached—usually 1–15 min.
8. Wash in absolute alcohol for 24 hr by changing the alcohol several times.
9. Pass through 2 xylene baths and, when cleared, mount in Canada balsam.

Steps 1, 2, 7, and 8 can be omitted, but they improve the quality of the clearing and the intensity of the staining.

Biotech Histochem Downloaded from informahealthcare.com by Karolinska Institutet University Library on 06/08/14
For personal use only.

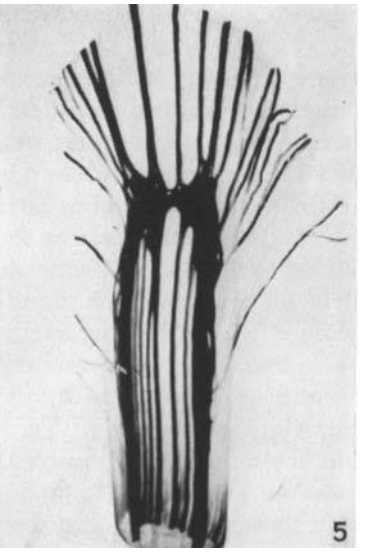

FIG. 5. *Eryngium maritimum* L., petiole; method No. 2 and fast green FCF. × 2
FIG. 6. *Juniperus chinensis* L., young leaves; method No. 2 and fast green FCF. × 38

If, after washing with water (step 4), the specimen does not appear to be sufficiently clear, or if the fuchsin has stained some of the nonlignified elements, it is possible to place it for several hours in a 5% solution of NaOH at 60° C and then take up the operations again as from step 4. This extra treatment has always been advantageous.

Method No. 2. This is suitable for rhododendron leaf, pine-needles and all plants that have a hard epidermis.

1. Place the sample in 95% alcohol for several days.
2. Stain for 24 hr in a 1% solution of basic fuchsin in 95% alcohol.
3. Wash in water until completely rehydrated.
4. Place the sample in a 15% solution of NaOH at 60° C until it clears completely (it should become pale yellow in color) by renewing the NaOH every 24 hr. About 3 days are required for young branches of *Juniperus* and *Larix*, and 5 days for leaves of the *Rhododendron* and *Abies*.
5. The remainder of the process is carried out as indicated in method No. 1, beginning with step 4.

Method No. 3. This can be used for the same purposes as method No. 2.

1. Place the sample in 95% alcohol for several days.
2. Rehydrate progressively.
3. Place in a 15% solution of NaOH at 60° C until completely cleared.
4. Stain with the following solution for 24 hr at 60° C: Dissolve 1 gm of basic fuchsin in 100 ml of boiling water; do not filter. After cooling, add 15 gm of NaOH.
5. Wash in water and finish as in method No. 1, steps 5–9.

Notes. Methods 2 and 3 can also be used for plants of a soft consistency, but

Biotech Histochem Downloaded from informahealthcare.com by Karolinska Institutet University Library on 06/08/14
For personal use only.

in this case the aqueous solutions or fuchsin solutions should contain only 10% NaOH (instead of 15%).

The staining of the lignified elements resists perfectly both 10% and 15% NaOH. It may happen that, immediately after the washing in water and no matter which method is used, the clearing seems inadequate (the parenchyma being brown, or the fuchsin has diffused). If so, it is always possible to improve the clearing by passing through a 10% or a 15% bath of NaOH at 60° C, and to start again: wash 12 hr in water, dehydrate, and finish the clearing sequence.

It is also possible to use water that is acidified with 5% HCl for washing; in which case, the red color of the lignified elements appears much more rapidly but does not attain the final intensity obtained with the other methods described.

It is convenient to place the specimens in a small Pyrex crystallization dish, and to eliminate the various liquids as completely as possible with a pipette. During the washing with water it is advisable to eliminate any possible deposits of fuchsin by means of a fine brush.

When samples are prepared for observation in a crystallization dish containing balsam, the best conditions are obtained only when the balsam is perfectly homogenous, therefore it is advisable to change it at least twice.

We sometimes stain the epidermis with fast green FCF in absolute alcohol between steps 8 and 9, and thus see the outline of the organ more easily.

REFERENCES

BRUNOTTE, C. 1900. Recherches embryogéniques et anatomiques sur quelques espèces d'Impatiens et de Tropaeolum. Thèse Fac. Sci. Paris.

DEBENHAM, E. M. 1939. A modified technique for the microscopic examination of the xylem of whole plants or plants organs. Ann. Bot., **N.S. 3,** 369–73.

FOSTER, A. S. 1949. Practical plant anatomy. 2nd ed. D. Van Nostrand Co., New York.

GABRIEL, C., and PELLISSIER, F. 1936. Une méthode d'éclaircissement et d'étude d'anatomie végétale par transparence. Bull. Soc. Bot. Fr., **83,** 816–19.

KASAPLIGH, B. 1951. Morphological and ontogenic studies of *Umbellaria californica* Nutt. and *Laurus nobilis* L. Univ. of California, Public. in Botany, **25** (1951–53), 115–239.

LEBEN, CURT. 1952. A method for clearing leaves. Phytopathology, **42,** 352.

PELLISSIER, F. 1939. Etude de l'ontogénie du système ligneux dés diverses phylles et de leur agencement dans la tige de Cucurbita pepo L. Thèse Fac. Sci. Paris.

PRAY, T. R. 1954. Foliar venation of angiosperms. I. Mature venation of *Liriodendron.* Amer. J. Bot., **41,** 663–70.

DE SLOOVER, J. 1958. Le sens longitudinal de la différenciation du procambium, du xylème et du phloème chez Coleus, Ligustrum, Anagallis et Taxus. La Cellule, LIX. n°1, 53–202.

WILSON, C. 1950. Vasculation of the stamen in the Melastomaceae, with some phyletic implications. Amer. J. Bot., **37,** 431–44.

Biotech Histochem Downloaded from informahealthcare.com by Karolinska Institutet University Library on 06/08/14
For personal use only.